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(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
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GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
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FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,  
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,  
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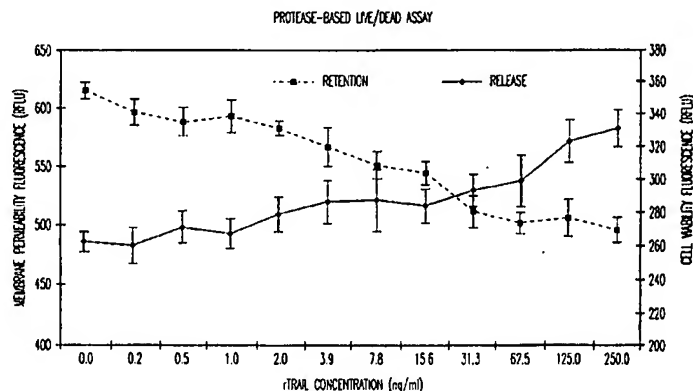
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For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: LUMINOGENIC AND NONLUMINOGENIC MULTIPLEX ASSAY



(57) Abstract: A method to detect the presence or amount of at least one molecule for an enzyme-mediated reaction in a multiplex luminogenic/nonluminogenic assay is provided.

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/US2005/002158

 A. CLASSIFICATION OF SUBJECT MATTER  
 INV. G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

 Minimum documentation searched (classification system followed by classification symbols)  
 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 602 657 B1 (BRONSTEIN IRENA Y ET AL) 5 August 2003 (2003-08-05) paragraphs [0013], [0030], [0055]; claims 11,35	1-14, 77-79
X	WO 01/46694 A (BIOSIGNAL PACKARD INC; JOLY, ERIK) 28 June 2001 (2001-06-28) page 11, lines 15-20; claims 9-20	1-14, 77-79
A	LIU J ET AL: "VISUALIZING AND QUANTIFYING PROTEIN SECRETION USING A RENILLA LUCIFERASE-GFP FUSION PROTEIN" LUMINESCENCE, WILEY, CHICHESTER, GB, vol. 15, no. 1, February 2000 (2000-02), pages 45-49, XP008001749 ISSN: 1522-7235 cited in the application abstract	1-14, 77-79
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*A\* document member of the same patent family

Date of the actual completion of the international search

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Weijland, Albert

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US2005/002158

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 314 805 A (HAUGLAND RICHARD P [US] ET AL) 24 May 1994 (1994-05-24) abstract -----	85-114
Y	US 2002/068316 A1 (RUST WILLIAM L [US] ET AL) 6 June 2002 (2002-06-06) abstract -----	85-114
Y	"APOPTOSIS INDUCERS AND THE ASSAY OF CASPASE ACTIVITY BIOMOL" FASEB JOURNAL, FED. OF AMERICAN SOC. FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD, US, vol. 12, no. 8, 24 April 1998 (1998-04-24), page A1488, XP002951634 ISSN: 0892-6638 abstract -----	85-114
A	EP 1 382 691 A2 (BAYER CROPS SCIENCE AG [DE]; MAX PLANCK GES ZUR FOERDERUNG D [DE] BAYER C) 21 January 2004 (2004-01-21) page 9, paragraph 2 -----	85-114
A	MONSEES T ET AL: "A NOVEL BIOLUMINOGENIC ASSAY FOR ALPHA-CHYMOTRYPSIN" JOURNAL OF BIOLUMINESCENCE AND CHEMILUMINESCENCE, WILEY AND SONS, CHICHESTER, GB, vol. 10, no. 4, July 1995 (1995-07), pages 213-218, XP009012508 ISSN: 0884-3996 abstract -----	85-114
A	KARLSSON J O ET AL: "Proteolytic activity in intact sheets of polarized epithelial cells as determined by a cell-permeable fluorogenic substrate." CELL BIOLOGY INTERNATIONAL 2000, vol. 24, no. 4, 2000, pages 235-243, XP002435585 ISSN: 1065-6995 abstract -----	85-114

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2005/002158

## Box II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 15-76, 80-114  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
85-114
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 15-76, 80-114

The present claims 1-14 and 77-79 relate to an extremely large number of possible methods/molecules/enzymes/ reaction mixtures/kits. Support and disclosure in the sense of Article 6 and 5 PCT is to be found however for only a very small proportion of the methods/molecules/enzymes/reaction mixtures claimed, see Example I. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 1-14 and 77-79 (PCT Guidelines 9.19 and 9.23).

Furthermore, the present claim 1 encompasses reactions defined by a desired result, i.e. "yields a... product"; this, with no link to how the "molecules" are detected. This is contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The search of claims 1-14 and 77-79 was restricted to the subject-matter as disclosed in present Example I.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 77-79

detecting in a sample a first and second molecule for an enzyme-mediated reaction yielding a luminogenic product and a nonluminogenic product and kit therefor.

2. claims: 15-48, 80-84

detecting in a sample an enzyme using a substrate of that enzyme, the reaction yielding a luminogenic product and kit therefor.

3. claims: 49-72

detecting in a sample a first and second enzyme using a first and second substrate yielding a luminogenic product and a nonluminogenic product.

4. claims: 73-76

detecting a fluorescent protein and a molecule in a sample containing cells, the reaction for the molecule yield a luminogenic product.

5. claims: 85-114

detecting live and/or dead cells using a cell permeant and a cell impermeant substrate and kit therefor.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2005/002158

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6602657	B1	05-08-2003	AU 732044 B2 12-04-2001
		AU 1350297 A 28-07-1997	
		CA 2241760 A1 10-07-1997	
		EP 0874913 A1 04-11-1998	
		JP 2000513563 T 17-10-2000	
		WO 9724460 A1 10-07-1997	
		US 6602658 B1 05-08-2003	
WO 0146694	A	28-06-2001	AU 2137101 A 03-07-2001
		US 2004214227 A1 28-10-2004	
US 5314805	A	24-05-1994	NONE
US 2002068316	A1	06-06-2002	NONE
EP 1382691	A2	21-01-2004	AT 343002 T 15-11-2006
		DE 10232902 A1 29-01-2004	
		DK 1382691 T3 19-02-2007	
		JP 2004049236 A 19-02-2004	
		US 2004053312 A1 18-03-2004	